PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 14 September 2000 (14.09.00)	
International application No. PCT/NL00/00058	**Applicant's or agent's file reference** P48485PC00
International filing date (day/month/year) 28 January 2000 (28.01.00)	**Priority date** (day/month/year) 28 January 1999 (28.01.99)
Applicant HEENEY, Jonathan, Luke	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

07 August 2000 (07.08.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer S. Mafla
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

NL0000058

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rul s 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
P48485PC00		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/NL 00/ 00058	28/01/2000	28/01/1999
Applicant		
STICHTING BIOMEDICAL PRIMATE RESEARCH CENTRE et al		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   COMPOSITION AND METHOD FOR OBTAINING SPECIFIC IMMUNISATION AGAINST ONE OR MORE ANTIGENS USING DIFFERENT RECOMBINANT VECTORS

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 00/00058

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 14-15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the vaccine compositions.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 00/00058

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K48/00 A61K39/00 A61K39/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IRVINE K R ET AL: "Enhancing efficacy of recombinant anticancer vaccines with prime/boost regimens that use two different vectors" JOURNAL OF THE NATIONAL CANCER INSTITUTE,US,US DEPT. OF HEALTH, EDICATIONAND WELFARE, PUBLIC HEALTH, vol. 89, no. 21, 5 November 1997 (1997-11-05), pages 1595-1601, XP002110478 ISSN: 0027-8874 page 1595 -page 1596 page 1599 -page 1600 --- -/--	1-17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 2000	15. 09. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mennessier, T

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 00/00058

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	LI S ET AL: "Priming with recombinant influenza virus followed by administration of recombinant vaccinia virus induces CD8+ T-cell-mediated protective immunity against malaria" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 90, no. 11, June 1993 (1993-06), pages 5214-5218, XP002110479 ISSN: 0027-8424 the whole document	1-17
X	WO 97 39771 A (US HEALTH ;CHAMBERLAIN RONALD S (US); IRVINE KARI R (US); ROSENBER) 30 October 1997 (1997-10-30) page 6 -page 19	1-17
P,X	ROSENWIRTH B ET AL: "An anti-HIV strategy combining chemotherapy and therapeutic vaccination." JOURNAL OF MEDICAL PRIMATOLOGY, (1999 AUG-OCT) 28 (4-5) 195-205. , XP000916685 page 195 -page 196	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 00/00058

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9739771 A	30-10-1997	AU 2678797 A	12-11-1997
		CA 2252406 A	30-10-1997

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

PRINS, A.W.
VEREENIGDE
Nieuwe Parklaan 97
2587 BN The Hague
PAYS-BAS

ONTVANGEN
07 MRT 2001
AMERSFOORT

Kopie v/naar

TERMIJN
28 FEB. 2001

Beantwoord voorl. def.

bericht gezonden aan

dd.

MAP

PCT

NRT2 28-7-2001

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)* 20.02.2001

Applicant's or agent's file reference
P48485PC00

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL00/00058	28/01/2000	28/01/1999

Applicant
STICHTING BIOMEDICAL PRIMATE RESEARCH CENTRE et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Pedersen, C Tel.+49 89 2399-8063

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P48485PC00		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL00/00058	28/01/2000	28/01/1999
International Patent Classification (IPC) or national classification and IPC A61K48/00		
Applicant STICHTING BIOMEDICAL PRIMATE RESEARCH CENTRE et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☐ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07/08/2000	20.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Mennessier, T Telephone No. +49 89 2399 8687

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL00/00058

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-24 as originally filed

Claims, No.:

1-17 as originally filed

Drawings, No.:

1-3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-17.

because:

☒ the said international application, or the said claims Nos. 14-16 (with respect to industrial applicability) relate to the following subject matter which does not require an international preliminary examination (*specify*): **see separate sheet**

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☒ the claims, or said claims Nos. 1-17 are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1. Comments with respect to item III

a) Industrial applicability

(i) Claims 14 and 15 (as a whole) and claim 16 (partly) relate to methods of treatment of the animal body by therapy, i.e., to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

(ii) For the assessment of the present claims 14-16 as well as claim 17 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

b) Unclarity and lack of support

(i) The extremely broad claimed subject-matter is not supported by the description and the claims contain a lot of unclarities, the defects being such that an accurately assessment of novelty and inventive step of the invention would be meaningless.

(ii) The claimed subject-matter is not supported by the description (an adequat support only exists if a person skilled in the art carrying out any of the embodiments which are claimed will re-obtain the advantageous technical effects referred to in the description) :

(1) The experimental part of the description reports assays carrying out in rhesus monkeys. Four groups of animals were immunized

and then boosted twice using 1, 2 or 3 of 3 series of recombinant vectors (DNA expression vectors, recombinant SFV vectors [rSVFs] and recombinant MVA vectors [rMVAs]). The DNA expression vectors were administered simultaneously as a pool of six "different" recombinant vectors, namely, vectors "pZH.UbgagPk", "pTH.UbpolPk", "pTH.UbnefPk", "pTH.tab", "pTH.rev" and "pND14-G1", each of them containing a sequence encoding a SIV-protein (gag, pol, nef, tab, rev and env, respectively). It would appear from pages 13-14 that also distinct rSFVs or rMVAs have been simultaneously administered (the precise vectors used are not described as such). It should be noted that the presence on any of the said vectors of a sequence encoding a proteinaceous molecule or any other molecule having an adjuvant function has not been mentioned. Two months after the second boost animals were challenged with a pathogenic cell-associated SIV stock "1XC". The number of protected animals was used as the criterium to compare the efficacy of the different regimens of vaccination.

(2) It appears it may be highly questionable whether the results obtained with such a complex experiment could be extrapolated to any other less or more sophisticated experiment. Would a person skilled in the art expect from these assays that animals primed with only DNA vectors encoding only one particular SIV protein, then boosted with only rSFV encoding the same antigen, and re-boosted with only rMVA also encoding the same antigen would favourably react as animals of group D as indicated in Table 18 on page 18? Would the introduction into the vector of a sequence encoding a molecule assumed to have an adjuvant function be efficient at improving the results presented in Table 1? It appears not to be possible to positively answer these questions.

(iii) The claims contain a lot of unclarities:

(1) The phrase "*one antigen of a virus causing temporary, or long*

lasting immune impairment" used in claim 1 is considered to be vague and indefinite due to the use of relative and imprecise terms.

(2) The repeated use of the term "*at least*" in claims 1 and 14 renders their subject-matter unclear.

(3) The term "*product*" used throughout the claims is vague and indefinite.

(4) In view of the statement found on page 4, lines 3-7, the term "*different*" used throughout the claims to feature the vectors appears to be vague and indefinite, insofar as two vectors which are not identical but similar will be regarded as different but, as being similar, will not be appropriate to perform the invention.

(5) The term "*vector*" also used throughout the claims is too vague. It should have been specified that the vectors to be used are recombinant vectors.

(6) It is not understandable how a vector as such could functions as an adjuvant (see claim 4).

(7) According to claims 1 and 14, the vaccine compositions would contain either the antigen(s) or a precursor thereof. This is totally unclear as the gist of the invention is precisely the use of compositions consisting of recombinant vectors encoding the antigen against which an immune response is expected.

2. Comments with respect to item VI

Should the priority would appear not to have been validly claimed the P-document which has been cited in the international search report (*Journal of Medical Primatology*, 28(4-5), 1999 August-October, 195-205) and to which one of the present inventors appears to have contributed should be taken into consideration

when assessing whether the claimed invention is new and involves an inventive step.

3. Comments with respect to item VIII

In view of the remarks made at point 1(b) above the whole set of claims is objected to under Article 6 PCT.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 48/00 | A2

(11) International Publication Number: WO 00/44410

(43) International Publication Date: 3 August 2000 (03.08.00)

(21) International Application Number: PCT/NL00/00058

(22) International Filing Date: 28 January 2000 (28.01.00)

(30) Priority Data:
99200256.8 28 January 1999 (28.01.99) EP

(71) Applicant *(for all designated States except US)*: STICHTING BIOMEDICAL PRIMATE RESEARCH CENTRE [NL/NL]; Lange Kleiweg 151, NL–2288 GJ Rijswijk (NL).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: HEENEY, Jonathan, Luke [CA/NL]; Vrijburgstraat 25, NL–2275 BX Voorburg (NL).

(74) Agent: OTTEVANGERS, S., U.; Vereenigde, Nieuwe Parklaan 97, NL–2587 BN The Hague (NL).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: PRODUCT AND METHOD FOR OBTAINING SPECIFIC IMMUNISATION WITH ONE OR MORE ANTIGENS

(57) Abstract

A large number of recombinant of viral and bacterial systems has been engineered as vectors to express foreign genes for vaccination and/or gene therapy. A common problem is the immune response to the vector itself. The presence of anti–vector immune responses may preclude sufficient priming or delivery if pre–existing immune responses are present, or impair optimal "boosting" upon subsequent immunisation or delivery. The invention provides means and methods for vaccinating an animal or a human to obtain therein an immune response against at least one antigen, comprising different vaccine compositions for sequential administration to said animal or said human, each containing at least said antigen or a precursor thereof, wherein said vaccine compositions differ from each other by the presence therein of a different vector.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
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BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

Title: Product and method for obtaining specific immunisation with one or more antigens.

FIELD OF THE INVENTION

The invention lies in the field of medicine. More particularly the invention relates to vaccines, vaccine compositions and vaccination strategies for obtaining improved immune protection against infectious diseases.

BACKGROUND OF THE INVENTION

The ultimate goal of developing prophylactic and/or therapeutic vaccines for a large number of infectious agents has been difficult to achieve due to the inability to induce optimal immune responses to the pathogen in a safe and effective manner. The previously tried and proven approaches of vaccination with whole killed or live attenuated viruses are either unsafe or ineffective for the remaining infectious diseases of major public health concern. To avoid possible safety problems it has been possible to develop protein based vaccines consisting of one or several individual viral proteins or epitopes thereof. These are derived from individual viral genes expressed in vitro and purified as individual subunits in the protein in the absence of genetic material. Recombinant subunit vaccine approaches have proven effective for certain pathogens such as Hepatitis B. However, for many applications subunit antigens have been unsuccessful due to expression/production difficulties, alteration of relevant immunological epitopes or marked variability of the pathogen requiring the continuous development, fermentation and purification of new antigens.

Recombinant live viral or bacterial vaccine vectors were developed as potential solutions to some of these problems. A replicating live virus or bacteria which does not cause disease has the potential to be used as a vector. Attenuated viruses such as adenovirus, poxvirus (i.e. vaccinia, MVA,

canary or fowlpox) or bacteria such as *E. coli*, are being developed and evaluated as live vectors. Due to their ability to replicate (in some cases in a limited fashion) in a host without serious side effects, makes them candidate to carry and express foreign genes as "vaccine" antigens. Recombinant vaccines have the advantage that they replicate in the host and thereby induce stronger immune responses than whole killed viruses or bacteria or subunit proteins. An additional advantage is that an immune response to an antigen encoded by said vector, may be improved by the stimulation of the immune system through the presence or the expression of additional proteins, for instance vector specific proteins for instance through providing adjuvant function. However, relatively few recombinant vector systems alone have been successful enough to be widely accepted for clinical use. Major problems other than safety have been pre-existing immunity in the case of vectors derived from infectious agents common in populations. Furthermore, subsequent immune responses against vector proteins themselves have created a further immunological barrier when more than one immunisation was required to boost responses to the recombinant vaccine antigen(s). One problem is that the immune system may mount an immune response against vector or vector encoded proteins together with an immune response against the antigen, designated the vaccination antigen, the immune response was intended to be directed toward in order to provide the host protection. The observation that the immune system may mount an immune response against a vector protein or a vector encoded protein creates a potential for competition for immune resources such as the availability of immune cells and/or cytokines, thereby lowering the desired response against vaccination proteins (see for example figure 1A). Another problem is the potential for more immunogenic antigens present in vector proteins or vector encoded proteins directing the immune response away from vaccination proteins. Additionally, immune responses against the vector eventually limit vector replication in the

host, thereby reducing the vectors intended purpose and effectiveness. A problem that specifically increases upon boosting of the immune response with the same or a similar vector or vector system. For instance, the use of different adenovirus serotypes comprising nucleic acid encoding similar vaccination proteins as vaccines is not optimal since the immune system will still be boosted against common antigens present in vector proteins and/or vector encoded proteins. A possible method to avoid this problem is to boost immune responses induced by the recombinant vectors with subunit protein. Several studies have shown that immune responses can be slightly improved by this method but that there is not a substantial improvement in the ability of the vaccine to protect from infection.

SUMMARY OF THE INVENTION

The present invention provides novel means and methods for obtaining a specific immune response in an individual or animal. The invention further provides means and methods for decreasing the negative effects of vector proteins and/or vector encoded proteins while leaving desired effects, such as an adjuvant effect of said proteins at least in part intact (see for a non-limiting example the scheme depicted in figure 1B).

In one aspect the invention provides a product for vaccinating an animal or a human to obtain therein an immune response against at least one antigen, comprising at least two different vaccine compositions for sequential administration to said animal or said human, each containing at least said antigen or a precursor thereof, wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector.

In another aspect the invention provides a method for vaccinating an animal or human to obtain therein an immune response against at least one antigen of a virus causing a

temporary, or long lasting immune impairment, comprising administering sequentially to said animal, at least two different vaccine compositions, each containing at least said antigen or a precursor thereof and wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector.

In yet another aspect the invention provides a use of an antigen, or a precursor thereof, for manufacturing a vaccine composition for vaccinating an animal or a human to obtain therein an immune response against said antigen, wherein said vaccine composition is administered sequentially with at least one other vaccine composition containing at least an immunogenic part, derivative and/or analogue of said antigen or antigen precursor, and a different vector.

DETAILED DESCRIPTION OF THE INVENTION.

In one aspect the invention provides a solution to circumvent the negative effects associated with repeated exposure of vector proteins or vector encoded proteins in a vaccination procedure or a vaccine composition. To study problems associated with amplification of an immune response against vector proteins and/or vector encoded proteins a strategy was developed in which the use of different vector systems, to consecutively deliver the same or related antigen(s), was evaluated. The potential existed not only to substantially boost immune responses to the recombinant antigen, but to tailor the nature of the immune responses by priming and then delivering subsequent boosts with different vector combinations or by delivering the vaccine vectors to different immunological sites and/or antigen presenting cell populations. Indeed, the ability to induce preferred type-1 or type-2 like T-helper responses or to additionally generate specific responses at mucosal and/or systemic sites can be foreseen with such an approach.

In one aspect the invention provides means and methods for vaccinating an animal or a human to obtain therein an immune response against at least one antigen of a virus causing a temporary, or long lasting immune impairment, comprising at least two different vaccine compositions for sequential administration to said animal or said human, each containing at least said antigen or a precursor thereof, wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector. A much better vaccination for such viruses is obtained with at least three different vaccine compositions wherein at least three of said vaccine compositions differ from each other by the presence therein of a different vector.

In another aspect the invention provides a product for vaccinating an animal or a human to obtain therein an immune response against an antigen comprising at least two different vaccine compositions for sequential administration to said animal or said human, each containing at least said antigen or a precursor thereof, wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector. An improved vaccination is obtained with at least three different vaccine compositions wherein at least three of said vaccine compositions differ from each other by the presence therein of a different vector. In a vaccination procedure comprising a serial administration to said animal of at least two vaccine compositions comprising at least said antigen or a precursor thereof and wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector, an amplification of an immune response against vector antigens that may be present in one or more of said vaccine compositions or that may be encoded by nucleic acid present in one or more of said vaccine compositions or both, is at least in part avoided in said animal. By at least in part avoiding said amplification of an immune response against vector antigens in said animal, potential masking of an

immune response against said antigen is at least in part prevented. One method of avoiding at least in part an amplification of an immune response against vector antigens in said animal is to avoid at least in part the presence of vector antigens in said animal during said vaccination procedure. This may be achieved for instance by avoiding the presence of vector antigens in at least one of said vaccine compositions or by avoiding at least in part, expression of vector antigens encoded by a nucleic acid in a vaccine composition, or both. Preferably, amplification of an immune response in said animal or human against vector antigens is at least in part prevented by using for said serial administration of vaccine compositions, vaccine compositions comprising different vectors. Another preferred method of avoiding amplification of an immune response against vector antigens in said vaccination procedure is to use at least one vaccine composition useful for avoiding the presence of vector antigens in said animal and at least one vaccine composition comprising a vector. Preferably, when more then one vaccine composition comprising a vector is used, said vector in said vaccine composition is essentially different.

A process for vaccinating an animal or human may be any vaccination process provided that said process utilises serial administration of vaccine compositions containing at least an antigen or a precursor thereof, against which said animal or human should at least in part be vaccinated. Vaccine compositions are preferably administered to said animal or human in an amount effective for eliciting an immune response in said animal or human.

Said antigen may be a complete protein or a part of a protein. Said antigen may also be a proteinaceous molecule, derived from nature or synthesised chemically.
In one embodiment of the invention said animal is a human.

In one embodiment the invention provides a product for vaccinating an animal or a human to obtain therein an immune response against at least one antigen, comprising at least

two different vaccine compositions for sequential administration to said animal or said human, each containing at least said antigen or a precursor thereof, wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector. Preferably said product comprises at least three of said compositions and wherein at least three of said vaccine compositions differ from each other by the presence therein of a different vector.

In one embodiment at least part of, said vector or a product thereof, functions as an adjuvant. An adjuvant in the context of the present invention is any molecule or combination of molecules, capable of modulating an immune response against said antigen. In one example an adjuvant has the capability to stimulate the immune system in said animal to elicit an immune response wherein said stimulation also stimulates the initiation or the amplification of an immune response against said antigen. In one example, an adjuvant is a classical adjuvant such as complete or incomplete freund adjuvant. In another example said adjuvant is a proteinaceous molecule immunologically different from said antigen, capable of eliciting an immune response in said animal or human.

Preferably said proteinaceous molecule comprises at least a functional part of a co-stimulatory molecule such as CD80, CD86, CD28, CD152, CD40 or CD40 ligand; of a cell-adhesion protein; of an immune response inhibitory protein; of an interleukin; of a major histocompatibility complex protein or of other proteins capable of modulating an immune response. An immune response may be modulated through at least in part inhibiting or preventing an immune response and/or at least in part inducing or enhancing an immune response.

In a preferred aspect of the invention vaccination is be performed together with a method for influencing at least in part immune system, for example in the direction of a preferred T helper 1 type of immune response or a more T

helper 2 type of immune response. It is now widely accepted that T cell-dependent immune responses can be classified on the basis of preferential activation and proliferation of two distinct subsets of $CD4^+$ T-cells termed T_H1 and T_H2. These subsets can be distinguished from each other by restricted cytokine secretion profiles. The T_H1 subset is a high producer of IFN-γ with limited or no production of IL-4, whereas the T_H2 phenotype typically shows high level production of both IL-4 and IL-5 with no substantial production of IFN-γ. Both phenotypes can develop from naive $CD4^+$ T cells and at present there is much evidence indicating that IL-12 and IFN-γ on the one hand and IL-4 on the other are key stimulatory cytokines in the differentiation process of pluripotent T_H0 precursor cells into T_H1 or T_H2 effector cells, respectively, *in vitro* and *in vivo*. Since IFN-γ inhibits the expansion and function of T_H2 effector cells and IL-4 has the opposite effect, the preferential expansion of either IFN-γ producing cells (pc) or IL-4 pc is indicative of whether an immune response mounts into a T_H1 or T_H2 direction. The cytokine environment, however, is not the only factor driving T_H lineage differentiation. Genetic background, antigen dose, route of antigen administration, type of antigen presenting cell (APC) and signalling via TCR and accessory molecules on T cells.

In a preferred aspect of the invention the immune system is directed toward a more T helper 1 or 2 type of immune response through using vectors with the property of modulating an immune response in one direction or the other. In a preferred aspect of the invention at least part of said adjuvant function comprises means for directing the immune system toward a more T helper 1 or 2 type of immune response.

Preferably through using vectors with the property of modulating an immune response in one direction or the other. Examples of vectors with the capacity to stimulate either a more T helper 1 or a more T helper 2 type of immune response or of delivery routes such as intramuscular or epidermal

delivery can be found in Robinson 1997, Vaccine 15:785-787; Sjolander et al 1997, Cell. Immunol. 177:69-76; Doc et al 1996, Proc. Natl. Acad. Sci. USA 93:8578-8583; Feltquate et al 1997, J. Immunol. 158:2278-2284; Pertmer et al 1996, J. Virol 70:6119-6125; Prayaga et al, Vaccine 15:1349-1352; Raz et al 1996, Proc. Natl. Acad. Sci. USA 93:5141-5145.

In a preferred aspect of the invention the immune system is induced to produce innate immune responses with adjuvant potential in the ability to induce local inflammatory responses. These responses include interferons, _-chemokines, and chemokines in general, capable of attracting antigen processing and presenting cells as well as certain lymphocyte populations for the production of additional specific immune responses. These innate type responses have different characteristics depending on the vector or DNA used and their specific immunomodulating characteristics, including such as encoded by CpG motifs, and as such, the site of immunisation. By using in a specific sequence different vectors encoding at least one common specific vaccine antigen, different kinds of desired protective vaccine responses may be generated and optimised for defence from a particular infectious agent. By combining different vector systems and delivering them at different or the same specific sites the desired vaccine effect at a particular site of entry (i.e. oral, nasal, enteric or urogenital) of the specific infectious agent.

In one aspect at least one of said vectors comprises antigen presenting cells, preferably engaged in vivo but also in vitro from said animal. Preferably said antigen presenting cells are dendritic cells. Preferably said antigen presenting cells present said antigen, or an immunogenic part, such as a peptide, or derivative and/or analogue thereof, in the context of major histocompatibility complex I or complex II.

In a preferred embodiment at least one of said compositions comprises as an antigen precursor a nucleic acid encoding at least one proteinaceous molecule for inducing and/or boosting an immune response against said antigen. In a

preferred embodiment said nucleic acid is capable of replicating in a cell of the animal or human being vaccinated. With the term boosting in this respect is meant amplifying an immune response such, that when said animal is exposed to said antigen after the amplification, the immune response to said antigen is increased in magnitude compared to before said amplification. Said proteinaceous molecule for inducing and/or boosting an immune response against said antigen may be said antigen or an immunogenic part, derivative or analogue thereof. Alternatively, antigen or an immunogenic part, derivative or analogue thereof may be encoded by a nucleic acid present in said vaccine composition.

In a preferred embodiment said antigen is an antigen encoded by a nucleic acid of a pathogen, preferably of a virus. In a particularly preferred embodiment said antigen is an antigen encoded by a virus which causes a temporary or long lasting immune impairment. For such viruses it has not been possible to devise a satisfactory vaccination strategy to completely protect from infection. The present invention is however, surprisingly suited to provide a satisfactory vaccination for viruses causing different degrees of immune impairment. Some vaccination is obtained using a product comprising at least two different vaccine compositions for sequential administration to said animal or said human, each containing at least said antigen or a precursor thereof, wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector. However, vaccination is substantially improved to provide substantial protection when at least three different vaccine compositions are used for sequential administration to said animal or said human, each containing at least said antigen or a precursor thereof, wherein at least three of said vaccine compositions differ from each other by the presence therein of a different vector.

For effective maintenance and further boosting of the vaccination it is preferred that the immune capacity of the vaccinated individual is boosted at intervals with a vaccine comprising yet another adjuvant. In a preferred embodiment said antigen encoded by a virus causing a temporary, or preferably long lasting immune impairement is an antigen of a lentivirus, another retrovirus, a hepatitis C virus, another flavivirus, a measles virus, another paramyxovirus or a Herpes Virus. In a preferred embodiment said antigen comprises at least an immunogenic part, derivative and/or analogue of a lentivirus *gag*, *pol*, *rev*, *tat*, *nef* or *env* protein or a combination thereof.

In a preferred embodiment at least part of said adjuvant function by a vector is provided by a nucleic acid which encodes at least one proteinaceous molecule capable of modulating an immune response. Preferably said nucleic acid is capable of replicating in a cell of the animal of the human being vaccinated. Preferably said proteinaceous molecule capable of modulating an immune response comprises a functional part of a co-stimulatory molecule such as CD80, CD86, CD28, CD152, CD40 or CD40 ligand; of a cell-adhesion protein; of an immune response inhibitory protein; of an interleukin; of a major histocompatibility complex protein or of other proteins capable of modulating an immune response.

In one embodiment the invention provides vaccine compositions wherein said vector is nucleic acid delivery vehicle comprising said nucleic acid. In a preferred embodiment said nucleic acid is capable of replicating in a cell of an animal or human being vaccinated. In a preferred embodiment said replicated nucleic acid has at least a limited capacity to spread to other cells of the host and start a new cycle of replication and antigen presentation and/or present adjuvant function. In a preferred embodiment said nucleic acid comprises nucleic acid of a Semliki Forest Virus, a poxvirus, a herpes virus and/or an adenovirus. In a

preferred embodiment said nucleic acid delivery vehicle is a Semliki Forest Virus particle, a pox virus particle, a herpes virus particle or an adenovirus particle.

In another embodiment the invention provides a method for vaccinating an animal to obtain therein an immune response against at least one antigen, comprising administering sequentially to said animal, at least two different vaccine compositions, each containing at least said antigen or a precursor thereof and wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector. Preferably said animal is a human.

In yet another embodiment the invention provides a use of a vaccine composition in a method or a product of the invention.

In yet another embodiment the invention provides a use of an antigen, or a precursor thereof, for manufacturing a vaccine composition for vaccinating an animal or a human to obtain therein an immune response against said antigen, wherein said vaccine composition is administered sequentially with at least one other vaccine composition containing at least an immunogenic part, derivative and/or analogue of said antigen or antigen precursor, and a different vector.

As proof of principle we undertook a vaccine efficacy study comparing one vector system alone, two different combinations of two different vector systems, and the use of three different vectors administered sequentially. All vectors used to immunise animals expressed similar SIV_{mac} antigens. Two months following the last immunisation animals were challenged intravenously with a highly pathogenic $SIV_{mac.1XC}$ inoculum and followed for evidence of protection.

EXAMPLES

MATERIALS AND METHODS

Study population

The study was carried out in outbred rhesus monkeys *(Macaca mulatta)*. Four groups of 4 animals and 1 group of 3 animals (19 rhesus monkeys in total) were studied. Each animal was identified by a unique animal number tattooed on the chest. The animals were derived from Indian genetic stock and purpose bred in captivity either in the USA (groups A, B, C, D, E) or the Netherlands (group F). Their age ranged from 2.5 to 3 years (groups A, B, C, D, E) or 10 to 11 years (group F). Their weights ranged between 2.7 and 3.9 kg (groups A, B, C, D, E) or 5.2 to 9.1 kg (group F). The animals were negative for SIV, STLV, SRV and had no previous immunosuppressive treatment. During the experiment all animals were housed separately in individual cages.

Three different vector systems were utilised, each containing the same genetic information for SIV *gag/pol*, *rev*, *tat*, *nef* and *env*. The vectors consisted of a bacterial plasmid based DNA expression vector, modified Vaccinia Virus Ankara (MVA) and Semliki Forest Virus (SFV). The first group (A) consisted of four animals immunised with SIV-MVA chimerics alone. Secondly, the immune responses obtained after immunisation with the DNA expression vectors and two boosters with either MVA-SIV (group B) or SFV-SIV (group C) vectors were compared to those obtained with a triple vector strategy; priming by immunisation first with DNA expression vectors, 1st booster with the MVA-SIV constructs, then 2nd booster with the SFV-SIV constructs (group D). The virus loads (by quantitative RNA PCR) were studied before and after virulent SIV challenge. Animals were challenged intravenously with a cell-associated SIV challenge stock (1XC).

In addition to the animals vaccinated *de novo*, 3 monkeys protected from a previous SIV vaccine study served as ìprotein primedî vector boost group (group F). They first received a boost with MVA-SIV, followed by SFV-SIV constructs.

Experimental design

Group A: One group of 4 animals immunised three times with MVA vectors expressing SIV *gag/pol*, *rev*, *tat*, *nef* and *env* administered intramuscularly.

Group B: One group of 4 animals immunised first intradermally with the DNA vectors expressing SIV *gag/pol*, *rev*, *tat*, *nef* and *env*, then boosted twice intramuscularly with MVA chimerics expressing similar SIV genes.

Group C: One group of 4 animals immunised with the DNA vectors expressing *gag/pol*, *rev*, *tat*, *nef* and *env* of SIV and boosted twice intravenously with SFV-SIV recombinant vectors expressing similar SIV genes.

Group D: One group of 4 animals vaccinated with the DNA expression vectors, boosted first with MVA-SIV chimerics and then with the SFV-SIV constructs.

Group E: One group of 4 control animals injected with empty DNA, and with the empty MVA and SFV vectors as infection controls.

Group F: One group of 3 animals which had proved to be protected from challenge in a previous study with a protein vaccine, then to be boosted first with the MVA-SIV chimerics, then with the SFV-SIV constructs.

DNA expression vector based vaccines

Vectors pTH.UbgagPk, pTH.UbpolPk, pTh.UbnefPk, pTH.tat, and pTH.rev express the *gag*, *pol*, *nef*, *tat* and *rev* genes of SIV_{macJ5} (Rud et al., 1994) under control of the human cytomegalovirus immediate-early (hCMV IE) enhancer/promotor (Hanke et al., 1998a). The vector pTH and cloning sites have been described previously (Hanke et al., 1998a; 1998b) in which the hCMV enhancer/promotor/intron A is cloned into the MIuI and HindIII sites and the individual SIVmacJ5 genes *tat* and cloned between HindIII and XbaI. Two vectors pTH.tat and pTH.rev contain the respective *rev* genes into the BamHI site without upstream Ub-R. The SIV_{macJ5} molecular clone was used as the source of these genes as previously described (Rud et al., 1994; Rhodes, A.D. et al., 1994; and Hanke et al., 1994). Vector pND14-G1 expresses the SIV_{mac239} envelope gp120 coding sequence under control of the hCMV IE enhancer/promotor and the simian D type retrovirus 1 (SRV-1) cis sequence was cloned between the gp120 gene and the BGH poly A/terminator region (Rhodes, G.H. et al., 1994; Indraccolo et al., 1998). All constructs contain the hCMV intron A sequence 5í of the expressed genes, in order to increase expression from the hCMV enhancer/promotor sequence, and carry the bovine growth hormone (BGH) polyA signal/terminator sequence. Each different DNA vector SIV construct was administered separately at a dose of 50 μg of DNA in 200 μl of saline with 1/2 of the volume injected into two separate sites intradermally.

SFV based vaccines

The SFV based vaccines used in this study express the *gag/pol*, *nef*, *tat*, *rev*, and *env* proteins of $SIV_{mac32H\ J5}$. The *gag/pol*, *env* and *nef* coding sequences and the *tat* and *rev* cDNAs from the pJ5 molecular clone of the SIV_{mac32H} proviral

genome (Rud et al., 1994; Rhodes, A.D. et al., 1994) were subcloned in the pSFV1 vector (Liljestrom and Garoff, 1991). The *gag/pol* coding sequences were obtained by PCR amplification to flank these genes by BamHI suitable for subcloning in the pSFV1 vector (Zhang et al., 1997). For packaging of recombinant SFV (rSFV) viral stocks a two-helper system was used (Smerdou and Liljestrom, 1999). Virus titres were determined by infection of BHK cells in limiting dilutions followed by indirect immunofluorescence using antibodies directed against relevant SIV proteins. Expression of the SIV antigens in infected BHK cells was also demonstrated by western blot and immunoprecipitation analysis of metabolically labelled BHK21 cells.

MVA based vaccines

Modified Vaccinia Ankara (MVA) (Sutter and Moss, 1995) recombinants in this study express the *gag/pol*, *nef*, *tat*, *rev*, and *env* genes of $SIV_{mac\ J5}$ (Rud et al., 1994; Rhodes, A.D. et al., 1994) under transcriptional control of P7.5 vaccinia virus early/late promotor (Sutter et al., 1994). Briefly, the *gag/pol*, *env* and *nef* coding sequences and the *tat* and *rev* cDNAs from the SIV_{macJ5} molecular clone (Rud et al., 1994; Rhodes et al., 1994) were subcloned in the MVA vector plasmid pIILzP7.5 at the SmaI site (Sutter and Moss, 1995; Sutter et al., 1994; and Seth et al., 1998) with the exception of *env* which was placed under control of a strong vaccinia vector promotor (Sutter et al., 1994). All of these reagents are stored and accessible through the NIBSC AIDS reagent repository, Potters Bar, U.K.

Vaccine challenge strain

The pathogenic, cell-associated SIV stock ($SIV_{mac32H.1XC}$) from primary, uncultured rhesus monkey PBMC "1XC", described previously (Niphuis et al., 1994), was used as the challenge

virus also described in a previous vaccine study (Heeney et al., 1994).

Administration of the vaccines

Rhesus monkeys were sedated with ketamin (10 mg/kg, prior to vaccine administration and bleedings. The vaccines were administered either intradermally (DNA vectors) or intramuscularly (MVA) or intravenously (SFV). In particular, 50 μg of each DNA expression vector in 200 μl of saline was administered per monkey, half of the volume injected into two separate sites. All immunisations with DNA were given twice at 12 week intervals followed by either MVA and/or SFV (see experimental design) at additional 12 week intervals.

Virus challenge and follow-up

All animals were challenged 2 months after the last immunisation with 50 MID_{50} of the pathogenic cell-associated SIV stock "1XC" administered by the intravenous route (volume: 1 ml/monkey) (Niphuis et al., 1994). Post-challenge readouts included quantification of plasma viral RNA as described previously (Ten Haaft et al., 1998), and assessment of CD4 T-cell numbers in peripheral blood.

Results and Discussion

To determine if protective immunity was obtained all animals were challenged with a highly pathogenic *in vivo* passaged rhesus PBMC stock of $SIV_{mac32H.1XC}$. As observed in figure 2E all of the control animals became readily infected (group E) with peak virus loads at two weeks reaching $5x10^6$ and $5x10^7$ RNA Eq/ml and remaining greater than $1x10^4$ RNA Eq/ml 12 weeks post-infection. All animals in group A, which received MVA-SIV constructs alone, also became infected (Table 1), although one animal had lower peak virus loads and a load lower than $1x10^4$ RNA Eq/ml by 6 weeks post-infection (Figure 2A). All animals in group B which received DNA-SIV

priming and MVA-SIV boosts also became infected (Table 1), with high virus loads persisting above pathogenic threshold levels (>1x10^5 viral RNA Eq/ml) after challenge. In group C one out of four animals was protected (Table 1) from infection, although those which became infected were not protected from virus load (Fig 2C).

Satisfactory protection was observed in animals which received three different vaccine vectors (Table 1) (Figure 2) with which protection from SIV challenge was obtained in 50% of the animals. Indeed, when subunit protein vaccinated animals which were previously protected from challenge were boosted 5 years later with a combination of two vectors (Group F, Table 1), vaccine protection was still observed in one out of three animals. Data post-infection revealed that immunisation did not sufficiently protect from virus load (Figure 2).

Improved protection against SIV infection was obtained when three vector systems were used (groups D, and F, Table 1). In group D, immunised with three different vector systems, protection against infection was found in 2 out of four immunised animals (Table 1, Figure 2D). Clearly, the use of one vector system alone for multiple immunisations was insufficient to protect from infection as in the case of MVA/SIV (group A) in this study (Table 1). This failure of protection from infection has been observed in other studies with SFV-SIV used alone for multiple immunisations (Mossman et al., 1996), although protection from acute symptoms (but not chronic disease) was suggested. A vaccine strategy using DNA priming and MVA boosting failed to protect immunised monkeys from infection (group B, Table 1). The use of DNA plus SFV to immunise showed limited promise in which one animal (group C, Table 1, Figure 2C) was protected from infection.

The best result against such potent challenges as the $SIV_{mac32H.1XC}$ used here was achieved with the use of three different vectors (group D, Table 1, Figure 2D). Further

proof was observed when the peripheral blood $CD4^+$ T-cells numbers were examined (Figure 3). The $SIV_{mac32H.1XC}$ used in this study causes a marked decline in $CD4^+$ T-cell numbers over time as observed in the control group (E) (Figure 3E) as well as other infected animals in this study. Notably, the animals which were protected from infection by the use of the triple vector strategy (animals BJV and CTC, group D) maintained normal $CD4^+$ T-cell levels while those of the infected animals declined. This was also noted in the protected animal (8645) in group F (Figure 3F) which has received a triple combination of a protein immunisation followed by MVA and SFV, further supporting this concept.

Through further refinement of this strategy, using combinations of different or divergent chimeric vectors, improved levels of vaccine protection are likely. Furthermore, optimisation of different combinations of vector systems delivered to different sites and populations of antigen presenting cells will lend this application to mucosal and/or combined mucosal/systemic vaccine strategies. It is envisioned that in addition differential modulation of immune responses (i.e. innate and specific such as type 1 vs type 2 T_H responses) and the induction of potent immunological memory will be possible using combinations of different vaccine vector systems.

Table 1. Experimental group and outcome

Group	"prime"	1st boost	2nd boost	protected
A	MVA-SIV	MVA-SIV	MVA-SIV	0/4
B	DNA-SIV	MVA-SIV	MVA-SIV	0/4
C	DNA-SIV	SFV-SIV	SFV-SIV	1/4
D	DNA-SIV	MVA-SIV	SFV-SIV	2/4
E	DNA	MVA	SFV	0/4
F	W.Virus protein "protected"	MVA-SIV	SFV-SIV	1/3

BRIEF DESCRIPTION OF THE DRAWINGS.

Figure 1:

A diagram comparing; (A) existing immunisation strategies with one delivery (i.e. vector) system; (B) the proposed combination of delivery (i.e., multiple vectors) systems. Immune responses to the desired Antigen are optimised and intensified with subsequent boosting with the combination strategy (B) as compared to conventional single delivery systems (A).

Figure 2

A comparison of plasma RNA virus loads in immunised and control animals which became infected after challenge with SIV. Figure 2A shows the virus loads in animals which had been immunised repeatedly with the same vector (3x MVA). Figure 2E shows the plasma virus loads in the control animals which were not immunised with any SIV antigen. Post-challenge virus loads for each of the other combination groups; B (DNA, 2x MVA), C (DNA, 2x SFV), D (DNA, MVA, SFV) and F (protein, MVA, SFV) respectively.

Figure 3

Comparison of $CD4^+$ T-cell levels following challenge per group. In infected animals $CD4^+$ T-cells declined as was especially evident in the control animals (E). The $CD4^+$ T-cell levels can be observed to remain at normal levels in protected animals, especially BJV and CTC in group D (D) which received the combination immunisation protocol. Groups depicted; A (3x MVA), B (DNA, 2x MVA), C (DNA, 2x SFV), D (DNA, MVA, SFV), E (controls), F (protein, MVA, SFV), respectively.

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Claims

1. A product for vaccinating an animal or a human to obtain therein an immune response against at least one antigen of a virus causing a temporary, or long lasting immune impairment, comprising at least two different vaccine compositions for sequential administration to said animal or said human, each containing at least said antigen or a precursor thereof, wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector.

2. A product for vaccinating an animal or a human to obtain therein an immune response against an antigen comprising at least two different vaccine compositions for sequential administration to said animal or said human, each containing at least said antigen or a precursor thereof, wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector

3. A product according to claim 1 or claim 2, wherein at least part of, said vector or a product thereof, functions as an adjuvant.

4. A product according to claim 3, wherein said adjuvant function directs the immune response toward a more T helper 1 type or a more T helper 2 type of response or both.

5. A product according to anyone of claims 1-4, wherein at least one of said compositions comprises as an antigen precursor a nucleic acid encoding at least one proteinaceous molecule for inducing and/or boosting an immune response against said antigen.

6. A product according to claim 5, wherein said proteinaceous molecule comprises said antigen, or an immunogenic part, derivative or analogue thereof.

7. A product according to anyone of claims 1-6, wherein said antigen is a part of or encoded by a virus, preferably a lentivirus or a hepatitis C virus.

8. A product according to anyone of claims 1-7, wherein said antigen comprises at least an immunogenic part, derivative and/or analogue of a lentivirus *gag*, *pol*, *rev*, *tat*, *nef* or *env* protein or a combination thereof.

9. A product according to anyone of claims 5-8, wherein a vector comprises a nucleic acid which encodes at least one proteinaceous molecule capable of modulating an immune response.

10. A product according to claim 9, wherein said proteinaceous molecule capable of modulating an immune response is a co-stimulatory protein, an immune response inhibitory protein, an interleukin, a major histocompatibility complex protein or a functional part, derivative and/or analogue thereof.

11. A product according to anyone of claims 5-10, wherein said vector is nucleic acid delivery vehicle comprising said nucleic acid.

12. A product according to anyone of claims 5-11, wherein said nucleic acid comprises nucleic acid of a Semliki Forest Virus, a poxvirus, a herpes virus and/or an adenovirus.

13. A product according to claim 11 or claim 12, wherein said nucleic acid delivery vehicle is a Semliki Forest Virus particle, a pox virus particle, a herpes virus particle or an adenovirus particle.

14. A method for vaccinating an animal to obtain therein an immune response against at least one antigen, comprising administering sequentially to said animal, at least two different vaccine compositions, each containing at least said antigen or a precursor thereof and wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector.

15. A method according to claim 14, wherein said animal is a human.

16. Use of a vaccine composition comprising at least one antigen or a precursor thereof, and a vector, in a product

according to anyone of claims 1-13, or a method according to claim 14 or claim 15.

17. Use of an antigen, or a precursor thereof, for manufacturing a vaccine composition for vaccinating an animal or a human to obtain therein an immune response against said antigen, wherein said vaccine composition is administered sequentially with at least one other vaccine composition containing at least an immunogenic part, derivative and/or analogue of said antigen or antigen precursor, and a different vector.

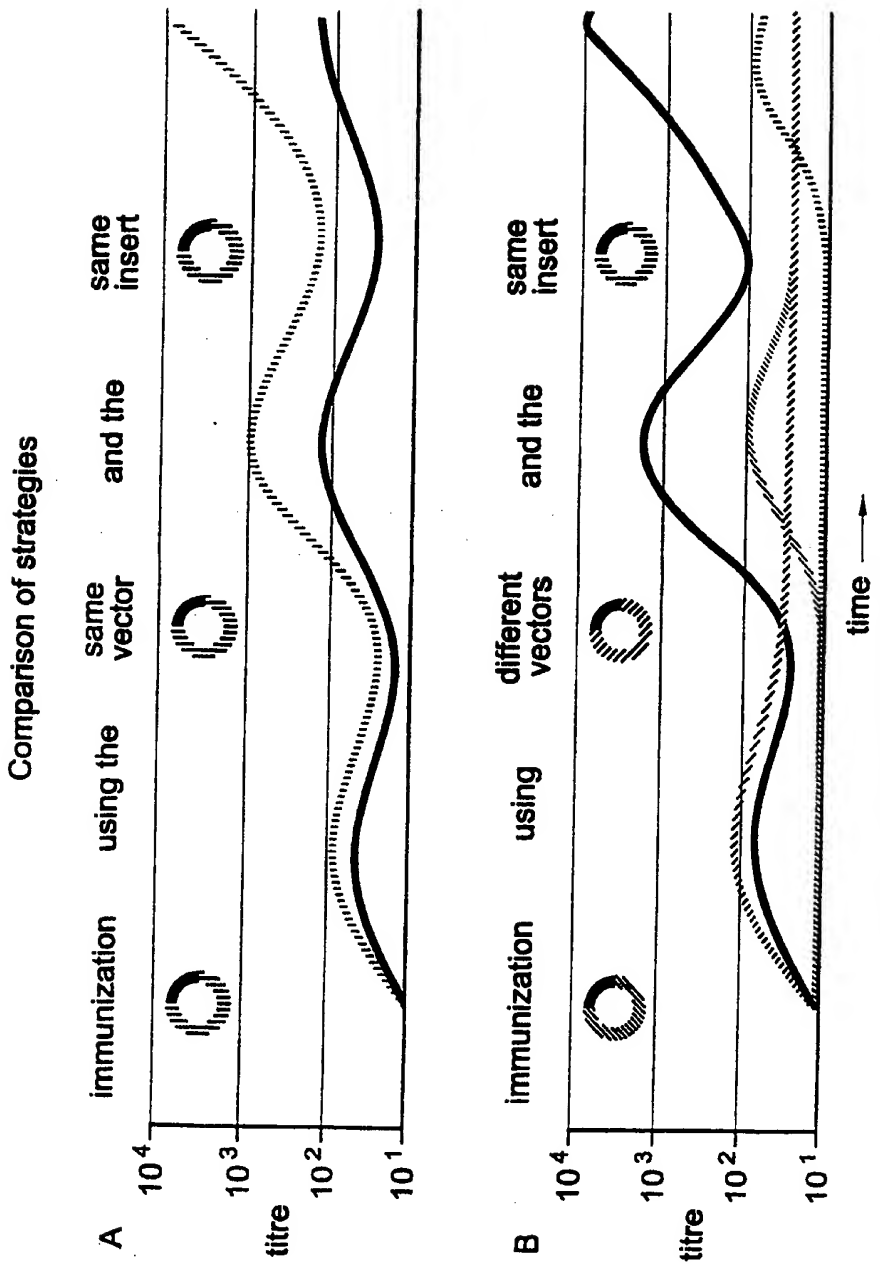
Comparison of strategies
A
immunization
using the
same
vector
and the
same
insert
titre
10^4
10^3
10^2
10^1
B
immunization
using
different
vectors
and the
same
insert
titre
time

Fig. 1

Plasma virus loads

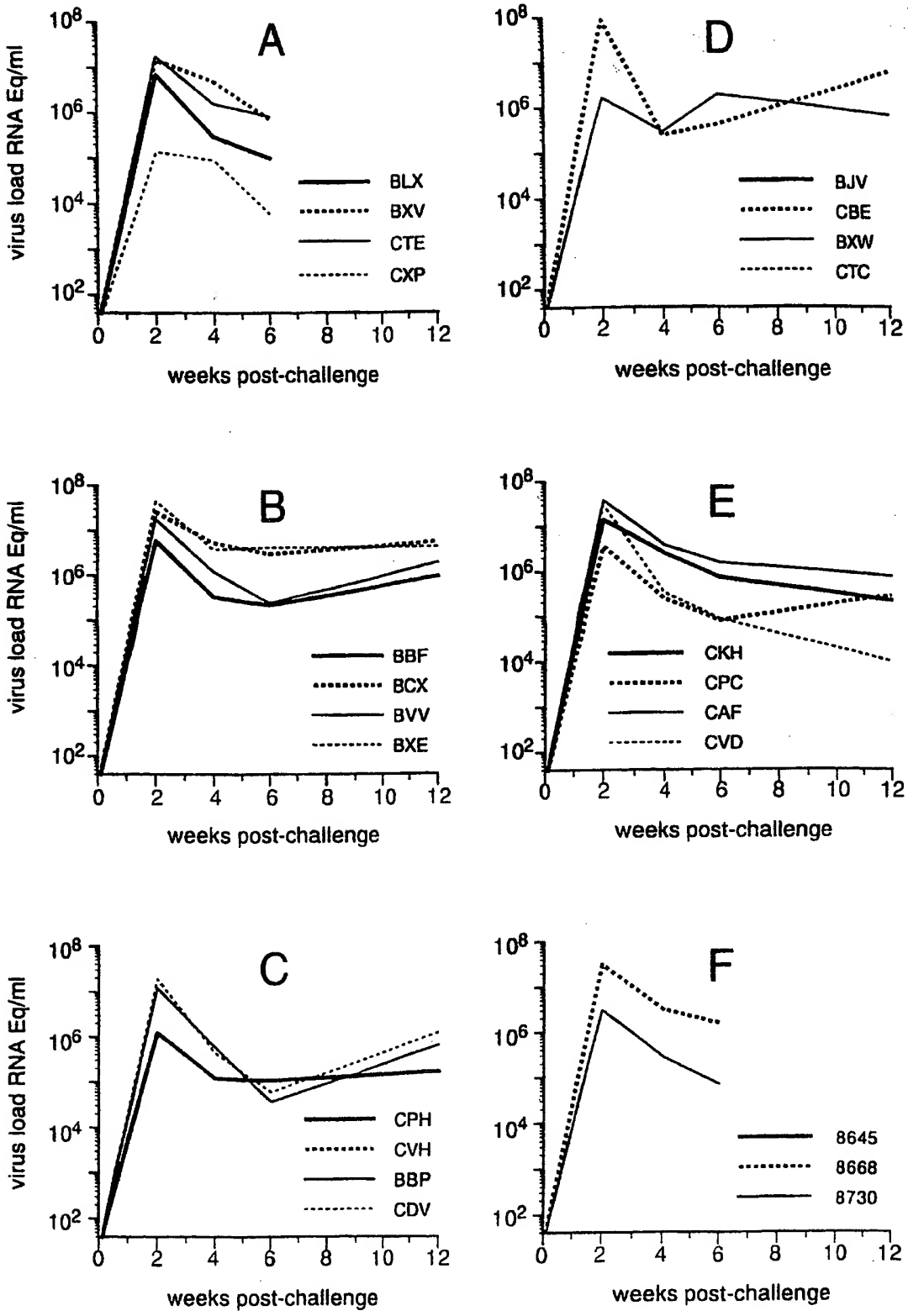

Fig.2

CD4+ T-c II levels

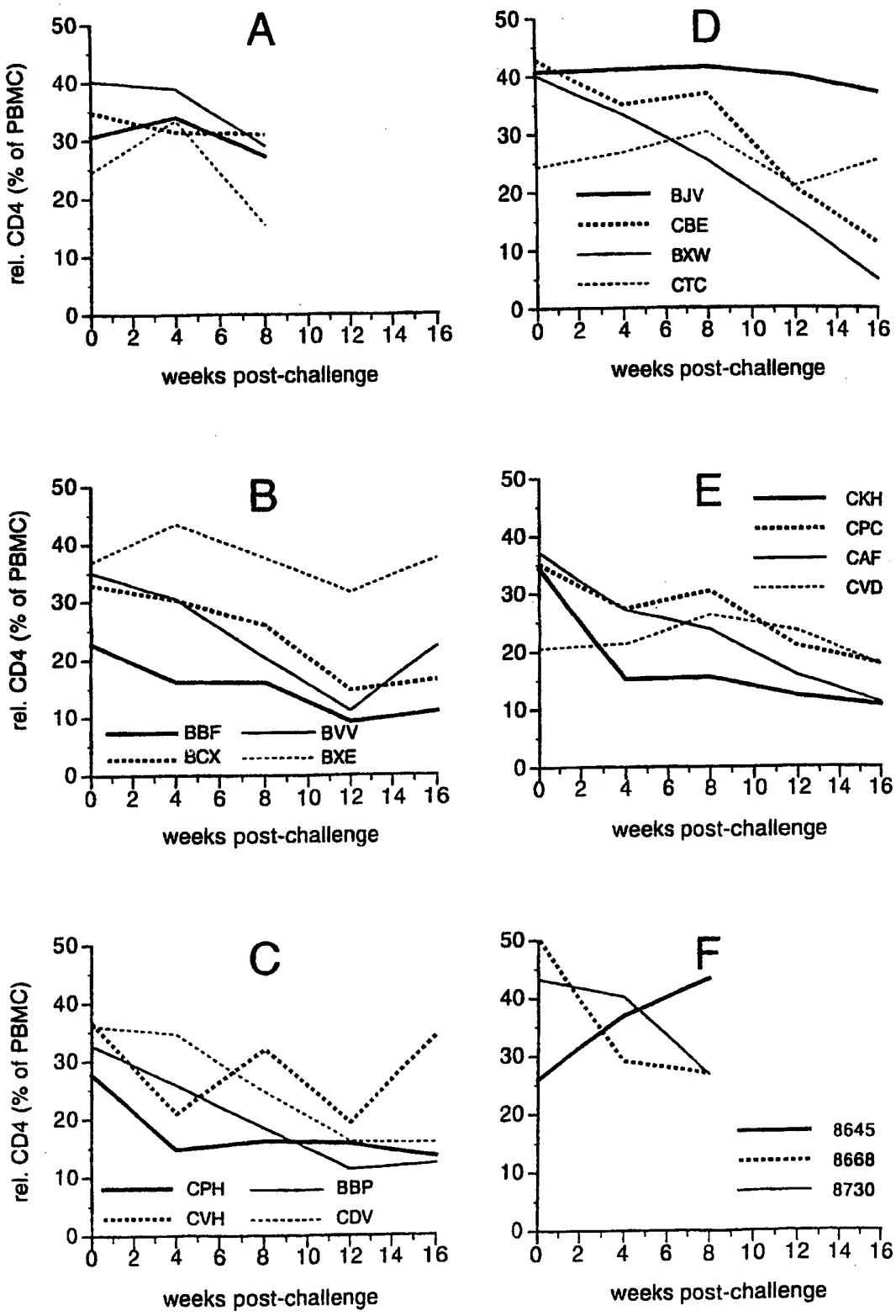

Fig.3

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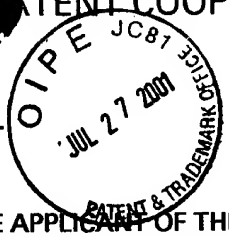

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NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Kopie in'naar

TERMIJN
15 AUG. 2000

Beantwoord vóór/ def MAP

Date of mailing (day/month/year) bericht gezonden aan
03 August 2000 (03.08.00)

Applicant's or agent's file reference
P48485PC00

IMPORTANT NOTICE

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/NL00/00058	28 January 2000 (28.01.00)	28 January 1999 (28.01.99)

Applicant
STICHTING BIOMEDICAL PRIMATE RESEARCH CENTRE et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
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   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
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   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
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REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

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(74) Agent: OTTEVANGERS, S., U.; Vereenigde, Nieuwe Parklaan 97, NL–2587 BN The Hague (NL).

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Published
Without international search report and to be republished upon receipt of that report.

(54) Title: PRODUCT AND METHOD FOR OBTAINING SPECIFIC IMMUNISATION WITH ONE OR MORE ANTIGENS

(57) Abstract

A large number of recombinant of viral and bacterial systems has been engineered as vectors to express foreign genes for vaccination and/or gene therapy. A common problem is the immune response to the vector itself. The presence of anti–vector immune responses may preclude sufficient priming or delivery if pre–existing immune responses are present, or impair optimal "boosting" upon subsequent immunisation or delivery. The invention provides means and methods for vaccinating an animal or a human to obtain therein an immune response against at least one antigen, comprising different vaccine compositions for sequential administration to said animal or said human, each containing at least said antigen or a precursor thereof, wherein said vaccine compositions differ from each other by the presence therein of a different vector.

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(71) Applicant *(for all designated States except US)*: **STICHTING BIOMEDICAL PRIMATE RESEARCH CENTRE** [NL/NL]; Lange Kleiweg 151, NL-2288 GJ Rijswijk (NL).

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(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Published:
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WO 00/44410 A3

(54) **Title:** COMPOSITION AND METHOD FOR OBTAINING SPECIFIC IMMUNISATION AGAINST ONE OR MORE ANTIGENS USING DIFFERENT RECOMBINANT VECTORS

(57) **Abstract:** A large number of recombinant of viral and bacterial systems has been engineered as vectors to express foreign genes for vaccination and/or gene therapy. A common problem is the immune response to the vector itself. The presence of anti-vector immune responses may preclude sufficient priming or delivery if pre-existing immune responses are present, or impair optimal "boosting" upon subsequent immunisation or delivery. The invention provides means and methods for vaccinating an animal or a human to obtain therein an immune response against at least one antigen, comprising different vaccine compositions for sequential administration to said animal or said human, each containing at least said antigen or a precursor thereof, wherein said vaccine compositions differ from each other by the presence therein of a different vector.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 00/00058

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K48/00 A61K39/00 A61K39/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IRVINE K R ET AL: "Enhancing efficacy of recombinant anticancer vaccines with prime/boost regimens that use two different vectors" JOURNAL OF THE NATIONAL CANCER INSTITUTE,US,US DEPT. OF HEALTH, EDICATIONAND WELFARE, PUBLIC HEALTH, vol. 89, no. 21, 5 November 1997 (1997-11-05), pages 1595-1601, XP002110478 ISSN: 0027-8874 page 1595 -page 1596 page 1599 -page 1600 --- -/--	1-17

[X] Further documents are listed in the continuation of box C.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 00/00058

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	LI S ET AL: "Priming with recombinant influenza virus followed by administration of recombinant vaccinia virus induces CD8+ T-cell-mediated protective immunity against malaria" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 90, no. 11, June 1993 (1993-06), pages 5214-5218, XP002110479 ISSN: 0027-8424 the whole document	1-17
X	WO 97 39771 A (US HEALTH ;CHAMBERLAIN RONALD S (US); IRVINE KARI R (US); ROSENBER) 30 October 1997 (1997-10-30) page 6 -page 19	1-17
P,X	ROSENWIRTH B ET AL: "An anti-HIV strategy combining chemotherapy and therapeutic vaccination." JOURNAL OF MEDICAL PRIMATOLOGY, (1999 AUG-OCT) 28 (4-5) 195-205. , XP000916685 page 195 -page 196	1-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 00/00058

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 14-15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the vaccine compositions.

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Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

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[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 00/00058

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9739771 A	30-10-1997	AU 2678797 A	12-11-1997
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